STUDIES IN THE METABOLISM OF PLANT CELLS

VII. THE QUANTITATIVE RELATION BETWEEN SALT ACCUMULATION AND SALT RESPIRATION

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[*Accepted for Publication December 2, 1947*]

Summary

This paper presents evidence of the quantitative relation between salt accumulation and salt respiration in plant cells.

Results of experiments on carrot tissue are given and similar results obtained by other workers with other tissues are discussed. Experiments with chlorides show that the rates of salt accumulation and of salt repiration are dependent on external concentration. When neither rate is limited by concentration, the number of molecules of salt accumulated is of the same order of magnitude as the number of electrons eliminated in salt respiration. This is based on the assumption that each oxygen molecule taken up in respiration requires four hydrogen ions and four electrons supplied by the respiratory carrier to form water.

These observations are consistent with Lundegardh's hypothesis that the electron carrier of respiration behaves as an anion carrier in accumulation, while the cations exchange with hydrogen ions. This hypothesis and the difficulties of testing it experimentally are discussed in detail; it is concluded that this hypothesis accords with most observations.

I. INTRODUCTION

The mechanism of the accumulation of salts in plant cells has been the subject of much investigation. Lundegardh (1945, 1946, 1947), in restating his hypothesis of salt accumulation, shows how the salt or anion respiration, by causing transport of anions, could provide a mechanism of accumulation. It has been shown (Robertson and Wilkins 1947) that the quantitative evidence available is not inconsistent with this hypothesis. In this paper the hypothesis will be discussed and the evidence will be given in detail.

The Lundegardh hypothesis rests on several observations:

(1) The total respiration of tissue is greater in salt solutions than in water. This increase in respiration rate, referred to as salt respiration, has been shown in wheat roots (Lundegardh 1940), potato tissue (Steward 1937), artichoke tissue (Steward and Berry 1934), carrot tissue (Robertson 1941), and barley roots (Hoagland and Broyer 1942).

(2) In dilute solutions, as salt respiration rate increases, the rate of salt accumulation increases.

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(3) Inhibitor evidence suggests that the cytochrome-cytochrome oxidase system is concerned in the salt respiration and in the mechanism of accumulation (Lundegardh 1940; Hoagland and Broyer 1942; Machlis 1944; Robertson and Turner 1945).

Lundegardh suggests:

(1) That electrons will be transported towards the surface of the cell by cytochrome in its reduced form, i.e. with ferrous iron.

(2) That the cytochrome, after loss of an electron by oxidation, has an excess positive charge and is then available to transport an anion with unit negative charge into the cell.

(3) That when this cytochrome is reduced with another electron, the anion will be left in the cell with the hydrogen liberated when the electron goes to the cytochrome.

(4) That cations will be available because they will enter the cell in exchange for hydrogen ions. Hydrogen ions are finally combined with oxygen and electrons to give water.

According to this theory, both ions of a salt are accumulated, provided that the rate of ion transport exceeds the rate of back diffusion or leakage from the cell. In its simplest terms, the suggested mechanism operates by transport of anions by an electron carrier (probably cytochrome) in the direction opposite to electrons and by exchange of cations for hydrogen ions; both electrons and hydrogen ions enter into the formation of water in the respiratory process. It is necessary to postulate that the path along which the electrons (and anions) move is separated in space from the path of the hydrogen ions (and entering cations). This involves some reasonable assumptions about the nature of the membrane.

In assessing the validity of the Lundegardh theory, the quantitative relationship between the salt or anion respiration and the salt accumulation is extremely important. Lundegardh (1940) has examined the variability of the coefficient, $k = \frac{\text{anion respiration}}{\text{anion accumulation}}$ (both in g. mol.), and concludes that "the coefficient k does not give evidence of any stoichiometrical relationship between the amount of absorbed anions and the amount of oxidized glucose . . . It cannot be expected that k should show any approximate degree of constancy unless the same plants at the same stage of development and the same temperature, etc., are always used." While, as Krogh (1942) points out, discrepancies in the constancy of this coefficient cannot be used as arguments against the conception of active transport of anions by the mechanism suggested, the evidence for the Lundegardh theory will be much stronger if it can be demonstrated that, under appropriate conditions, a stoichiometrical relationship exists and if the departures from the relationship can be explained.

If the Lundegardh theory is valid, the maximum rate of accumulation of which the cell is capable should occur when each electron leaving via the cytochrome system is exchanged for an anion from the external solution. If the respiration is proceeding by a cytochrome system, all the molecular oxygen concerned in the process is combined to form water, and each molecule of oxygen therefore requires four electrons and four hydrogen ions (Ball 1944). With the normal relationship of sugar breakdown, oxygen utilization and carbon dioxide production, the equation for respiration must be written:

$$C_6H_{12}O_6 + 6O_2 + 6H_2O \rightarrow 6CO_2 + 12H_2O.$$

The maximum rate of salt accumulation should therefore be 4 g. mol. monovalent salt accumulated per g. mol. oxygen utilized or $\frac{\text{salt accumulation}}{\text{salt respiration}} = 4$. This is the reciprocal of the ratio used by Lundegardh.

II. Materials and Methods

The materials and methods used in the collection of the data presented in this paper were similar to those described in earlier papers in this series (Robertson 1941; Robertson and Turner 1945).

The material was xylem parenchyma of carrot, *Daucus carota* L., obtained from various sources. Tissue for the experiments was prepared by cutting into discs and washing for long periods in aerated distilled water. Because of the effects of cutting, the experiments from which the results are taken included no tissue washed for less than 120 hours. In most experiments the period from cutting was between 120 and 350 hours.

The respiration was measured as oxygen uptake by standard Warburg technique or by carbon dioxide output in a continuous gas stream technique. In both techniques, aeration of the tissue was adequate to maintain the maximum aerobic respiration. A number of experiments have shown that the R.Q. of carrot tissue can be taken as unity, both in water and in solutions of monovalent salts.

Accumulation rate was measured by following either the conductivity changes or the changes in chloride concentration in the solutions surrounding the tissue. When respiration was measured by the Warburg technique, replicate sets of tissue for accumulation determinations were held in flasks in the thermostat and attached to the Warburg shaker to ensure aeration. When the respiration was measured by the continuous gas stream technique, the accumulation rate was followed by withdrawing samples of solution from the tissue vessels without interruption of the gas stream. In some experiments both chloride and conductivity methods were used and the accumulation rates so determined were in good agreement. When the conductivity method is used, it is important to note that the measurements represent the number of ions accumulated, as distinct from the total number of ions absorbed. The total number includes those entering by exchange for other ions. The agreement between chloride determinations and conductivity determinations indicated that little chloride, usually not exceeding 10 per cent., was being exchanged for other ions.

It has been pointed out previously (Robertson 1944; Robertson and Turner 1945) that there is a rapid uptake of ions immediately after the addition of salt. This initial uptake has been interpreted as a physical equilibration of the internal and external concentrations and is to be further investigated. After the internal concentration of salt approximates to the external concentration, accumulation proceeds against the concentration gradient, the rate decreasing slowly as the internal concentration increases (Robertson 1941).

The values for salt respiration — the difference between the steady rate in salt solution and the steady rate in water — are taken one and a half to two hours after adding the salt. This salt respiration in well-washed carrot tissue is usually, though not always, equivalent to the cyanide-sensitive respiration, the distilled water respiration being cyanide insensitive. Values for salt accumulation rate were taken over a few hours after the initial uptake period which lasts about half an hour after adding the salt. This is the maximum rate of accumulation and is steady over this period. The salt respiration rate is expressed as g. mol. O_2 or CO_2/hr./g. fresh wt. and the salt accumulation as g. mol. salt accumulated /hr./g. fresh wt.

Most experiments were carried out at 25°C.; some experiments were done at a different temperature (21°C. was the lowest), and the results have been corrected to the value expected at 25°C. assuming a Q_{10} of 2 (Robertson 1944).

III. Results

(*a*) *Experiments on Carrot Tissue*

The results of a number of experiments with different concentrations of potassium chloride are given in Table 1 and in Figure 1.

Table 1

Concentration of Salt (molar.)	No. of Observations	Mean Ratio $\frac{\text{Accumulation Rate}}{\text{Respiration Rate}}$	Method
0.00063	2	0.44 ± 0.16	Conductivity
0.00125	2	1.60 ± 0.73	"
0.0025	1	2.09	"
0.005	6	2.26 ± 0.41	"
0.01	19	2.88 ± 0.84	17 Conductivity 2 Chloride
0.02	16	2.78 ± 0.62	12 Conductivity 4 Chloride
0.03	4	3.00 ± 0.23	Conductivity
0.04	6	3.17 ± 0.79	5 Conductivity 1 Chloride
0.05	8	3.39 ± 0.70	Conductivity
0.06	4	3.38 ± 0.37	"

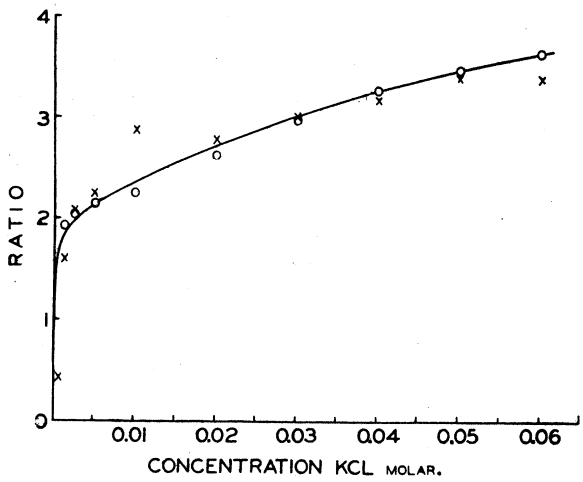

Fig. 1.—The ratio $\frac{\text{salt accumulation in g. mol.}}{\text{salt respiration in g. mol.}}$ for carrot tissue in dilute solutions of potassium chloride.

Crosses, mean ratios from Table 1; circles, ratios from lines of best fit in Figure 2.

These results were collected in the course of experiments over a long period and most of them were not specially designed to study this ratio; consequently different sets of tissue were used with the different concentrations. The results of experiments with potassium chloride, designed to test the relationship, are given in detail in Table 2.

TABLE 2

Stock of Carrots	No. of Exp.	Hours from Cutting	Concn. of Salt (molar.)	Mean Salt Respiration (g. mol./g./hr.) (x10^5)	No. of Replicates	Mean Salt Accumulation (g. mol./g./hr.) (x10^5)	No. of Replicates	Ratio
A	1	168	0.01	0.074	2	0.236	2	3.19
		168	0.03	0.109	2	0.343	2	3.15
		168	0.05	0.114	4	0.386	4	3.39
	2	216	0.01	0.079	2	0.214	2	2.71
		216	0.03	0.100	2	0.276	2	2.76
		216	0.05	0.113	4	0.388	4	3.43
	3	312	0.01	0.114	2	0.215	2	1.89
		312	0.03	0.091	2	0.284	2	3.12
		312	0.05	0.091	4	0.353	4	3.88
B	1	144	0.01	0.055	1	0.236	1	4.29
		144	0.03	0.108	1	0.349	1	3.23
		144	0.05	0.109	1	0.435	1	3.99
	2	168	0.01	0.116	1	0.244	1	2.10
		168	0.03	0.142	1	0.329	1	2.32
		168	0.05	0.108	1	0.337	1	3.12

(TABLE *continued over page*)

TABLE 2 (continued)

Stock of Carrots	No. of Exp.	Hours from Cutting	Concn. of Salt (molar.)	Mean Salt Respiration (g. mol./g./hr.) (x10^5)	No. of Replicates	Mean Salt Accumulation (g. mol./g./hr.) (x10^5)	No. of Replicates	Ratio
C	1	144	0.005	0.094	2	0.190	2	2.02
		144	0.01	0.117	2	0.215	2	1.84
		144	0.02	0.106	2	0.250	2	2.36
		144	0.04	0.074	2	0.300	2	4.05
		144	0.05	0.098	2	0.325	2	3.32
		144	0.06	0.100	2	0.335	2	3.35
	2	192	0.005	0.083	2	0.190	2	2.29
		192	0.01	0.128	2	0.270	2	2.11
		192	0.02	0.125	2	0.350	2	2.80
		192	0.04	0.139	2	0.365	2	2.63
		192	0.05	0.106	2	0.350	2	3.30
		192	0.06	0.120	2	0.360	2	3.00
	3	312	0.005	0.090	2	0.220	2	2.44
		312	0.01	0.114	2	0.320	2	2.81
		312	0.02	0.114	2	0.430	2	3.77
		312	0.04	0.144	2	0.430	2	2.99
		312	0.05	0.153	2	0.410	2	2.68
		312	0.06	0.128	2	0.435	2	3.40

These results were obtained with dilute solutions; since the ratio was increasing with increasing concentration, an experiment with tissue in higher concentrations was carried out on another stock of tissue. Results are given in Table 3.

TABLE 3

No. of Exp.	Hours from Cutting	Concn. of Salt (molar.)	Salt Respiration (g. mol./g./hr.) (x10^5)	No. of Replicates	Salt Accumulation (g. mol./g./hr. (x10^5)	No. of Replicates	Ratio
1	120	0.01	0.086	2	0.180	2	2.09
		0.04	0.110	2	0.290	1	2.64
		0.07	0.106	2	0.280	2	2.64
		0.10	0.108	2	0.280	2	2.59
		0.13	0.106	2	0.235	2	2.22
		0.16	0.125	2	0.280	1	2.24
2	168	0.01	0.090	2	0.220	2	2.44
		0.04	0.106	2	0.390	2	3.68
		0.07	0.110	2	0.345	2	3.14
		0.10	0.147	2	0.335	2	2.28
		0.13	0.104	2	0.340	2	3.27
		0.16	0.106	1	0.330	2	3.11

The salt respiration and salt accumulation rates are plotted against concentration in Figures 2 and 3, and the lines of best fit (by inspection) are drawn. The ratios from the smooth curves in Figures 2 and 3 are plotted in Figure 4 (Curves B and C).

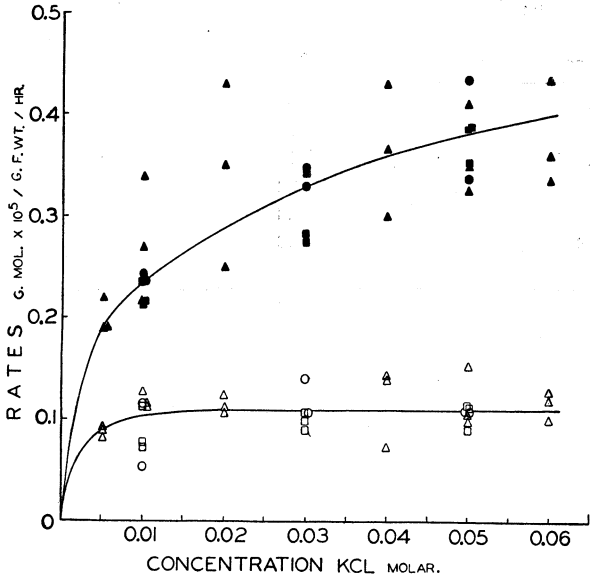

Fig. 2.—Rates of salt respiration and of salt accumulation for carrot tissue in dilute solutions of potassium chloride.

Data from Table 2: open symbols, salt respiration; solid symbols, salt accumulation; squares, stock of carrots A; circles, stock of carrots B; triangles, stock of carrots C.

A limited number of experiments with other salts, using the conductivity technique show that ratios for dilute solutions of other halides are of the same order of magnitude as those for potassium chloride. Results for an experiment in which dilute solutions of potassium chloride and calcium chloride were used on replicate sets of tissue are given in Table 4.

TABLE 4

Concentration of Salt (molar.)	No. of Observations	Salt Respiration (g. mol./g./hr.) ($\times 10^5$)		Chloride Accumulation (g. mol./g./hr. ($\times 10^5$)	
		KCl	$CaCl_2$	KCl	$CaCl_2$
0.00063	2	0.059	0.069	0.023	0.054
0.00125	2	0.062	0.080	0.091	0.102
0.0025	1	0.061	0.079	0.128	0.256
0.005	1	0.070	0.074	0.209	0.268
0.01	4	0.108	0.116	0.319	0.240
0.02	1	0.057	0.068	0.336	0.298

The ratios calculated from the curves of best fit to these respiration and accumulation data are given in Table 5.

TABLE 5

Concentration of Salt (normal.)	Ratio	
	KCl	$CaCl_2$
0.00063	0.7	0.7
0.00125	1.2	1.0
0.0025	1.9	1.6
0.005	2.8	2.6
0.01	4.0	3.4
0.02	4.2	3.4
0.04	---	3.4

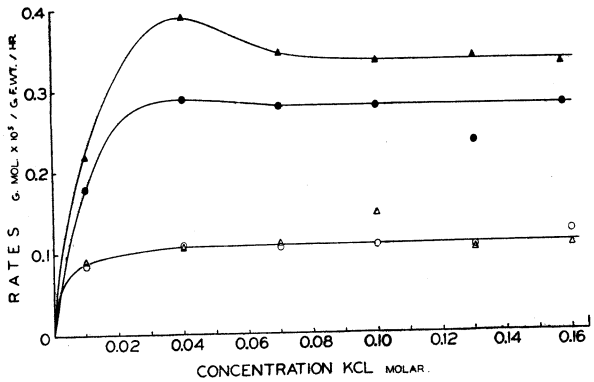

Fig. 3.—Rates of salt respiration and of salt accumulation for carrot tissue in concentrated solutions of potassium chloride.

Data from Table 3: open symbols, salt respiration; solid symbols, salt accumulation; circles, 120 hours from cutting; triangles, 168 hours from cutting.

More concentrated solutions were used in another experiment, and the results are given in Table 6.

TABLE 6

Concentration of Salt (normal.)	Salt Respiration g. mol./g./hr. (x10⁵)		Chloride Accumulation g. mol./g./hr. (x10⁵)		Ratio*	
	KCl	$CaCl_2$	KCl	$CaCl_2$	KCl	$CaCl_2$
0.01	0.090	0.083	0.240	0.180	2.67	1.60
0.04	0.098	0.110	0.360	0.140	3.30	1.57
0.07	0.118	0.138	0.320	0.170	3.34	1.51
0.10	—	0.122	0.390	0.230	3.36	1.48
0.13	0.114	0.126	0.390	0.180	3.36	1.48
0.16	0.114	0.131	0.380	0.200	3.36	1.48

*The ratios given in this table are calculated from the curves of best fit to the salt respiration and accumulation data.

In other experiments, the effects of 0.01M potassium chloride, lithium chloride, and sodium chloride were compared in replicate sets of tissue. Results are given in Table 7.

TABLE 7

No. of Exp.	Salt Respiration (g. mol./g./hr.) (x10^5)			Salt Accumulation (g. mol./g./hr.) (x10^5)			Ratios		
	KCl	NaCl	LiCl	KCl	NaCl	LiCl	KCl	NaCl	LiCl
1	0.065	0.155	0.109	0.270	0.299	0.175	4.15	1.93	1.79
2	0.106	0.100	0.057	0.203	0.255	0.136	1.91	2.55	2.39
3	0.121	0.095	0.060	0.196	0.169	0.116	1.62	1.78	1.93
4	0.057	0.080	0.054	0.336	0.310	0.264	5.90	3.88	4.89
5	0.042	0.071	0.054	0.175	0.198	0.128	4.17	2.79	2.37

In some experiments, potassium chloride, potassium bromide, and potassium iodide were applied to replicate sets of tissue. The results are given in Table 8.

TABLE 8

Concentration of Salt (molar.)	No. of Observations	Ratio KCl	KBr	KI
0.01	3	2.25 ± 0.45		
	2		1.86 ± 0.11	
	3			1.15 ± 0.11

The differences between potassium chloride and the other salts is not significant but the difference between potassium bromide and potassium iodide is significant at the 2 per cent. level.

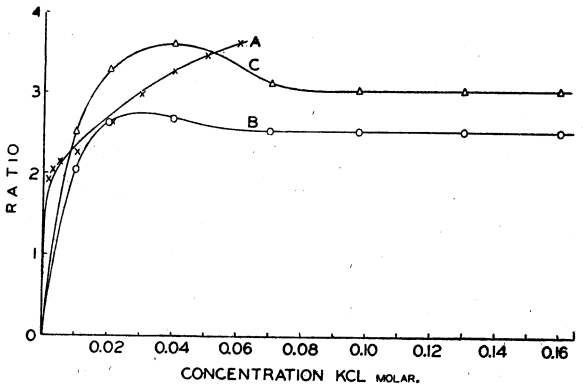

Fig. 4.—The ratio $\frac{\text{salt accumulation in g. mol.}}{\text{salt respiration in g. mol.}}$ for carrot tissue in concentrated solutions of potassium chloride.

Ratios from lines of best fit in Figure 3; circles, 120 hours from cutting; triangles, 168 hours from cutting; crosses, ratios from lines of best fit in Figure 2 for comparison.

(*b*) *Experiments on Other Tissues*

Some data were collected on beet tissue (Robertson, Turner, and Wilkins, unpublished data) and on barley roots (Milthorpe and Robertson, unpublished data). The tissue was not supplied with a range of salt concentrations but the results are worth recording here because the ratios are of the same order of magnitude as for comparable concentrations with carrot. In all experiments the salt respiration was taken as the difference between the total respiration and the respiration in water. The ratios are given in Table 9.

TABLE 9

Tissue	Salt	Concentration (molar.)	No. of Observations	Mean Ratio
Beet	KCl	0.01	5	2.63
		0.02	11	3.46
Barley Roots	KCl	0.01	10	1.29

(*c*) *Experiments of Other Workers*

Few data in the literature on salt accumulation, apart from those given by Lundegardh, are suitable for assessing the salt accumulation/salt respiration ratio. The ratio cannot be obtained from published experimental data for two principal reasons: (i) the data are insufficient to allow calculation of the difference between total respiration rate in salt and the respiration in water; and (ii) there is a lack of information about the initial or maximum accumulation rate in those instances where the rate changes markedly with time as internal concentration increases.

From the results summarized in Lundegardh's 1940 and 1945 papers, and collected from papers of Lundegardh and Burstrom, the salt respiration can be estimated in several different ways, and since all the experiments were over short periods (up to six hours), the salt accumulation is probably fairly near to its maximum. In estimating the salt respiration several different methods were used: (i) in some results the difference between the rate in salt solution and the rate in water can be obtained; (ii) in others the rate of salt respiration at different concentrations is given and an extrapolation to give the rate in zero salt concentration is possible; this assumes that the relationship of respiration to concentration is linear over the range of concentration used; and (iii) Lundegardh and Burstrom showed that the anion respiration is cyanide-sensitive, and in some results the difference between the total respiration rate in salt and the respiration in cyanide can be taken. Results obtained from Lundegardh's work are given in Table 10.

TABLE 10

Reference	Tissue	Method of Obtaining Salt Respiration Rate	No. of Observations	Salt	Accumulation / Respiration
Lundegardh (1940, p. 314)	Wheat	(ii)	3	0.002M KCl	0.31
			1	0.004M KCl	0.34
Lundegardh (1945, p. 34)	„	(ii)	3	0.002M KCl	0.34
Lundegardh (1945, p. 34)	„	(i)	3	0.001M KCl	0.70
Lundegardh (1945, p. 35)	„	(i)	1	0.001M KCl	0.24
		(i)	1	0.002M KCl	0.32
Lundegardh (1945, p. 34)	„	(iii)	2	0.002M KCl	0.46
Lundegardh (1940, p. 348)	Barley	(ii)	1	0.001M KCl	0.47
			1	0.004M KCl	1.60

Other salts investigated by Lundegardh, though giving different ratios, gave results of the same order of magnitude.

Some other workers have published results suitable for the calculation of the ratios; these are given in Table 11.

TABLE 11

Reference	Tissue	Method of Obtaining Salt Respiration Rate	No. of Observations	Salt	Accumulation / Respiration
Steward (1933, p. 208)	Potato	(i)	1	0.000468M KBr	1.4
			1	0.00468M KBr	2.0
			1	0.0468M KBr	3.2
Steward and Berry (1934)	Artichoke	(i)	1	0.00075M KBr	2.4
Van Eijk (1939)	Aster	(i)	3	0.171M NaCl	2.3
			1	0.342M NaCl	2.4

IV. DISCUSSION

(*a*) *Restatement of the Lundegardh Theory*

For purposes of discussion it is convenient to make a modified statement of the Lundegardh theory and this is best done by reference to a diagram (Fig. 5).

Without introducing any complicating assumptions regarding membranes, the cyclic system, fed by electrons, which is responsible for the anion transport, may be considered. When the hydrogen atom liberated by the dehydrogenase

stage of respiration reaches the cytochrome system, the electron is picked up by the cytochrome and the hydrogen ion is freed. The electron reduces the ferri-cytochrome (represented by $(Fe^{+++}$), and the resulting ferro-cytochrome (represented by $(Fe^{++}$) may be pictured as moving towards the oxidase. At the oxidase, the electron is combined with oxygen, a hydrogen ion is picked up from the environment and attached to oxygen, and the cytochrome is oxidized to the ferri-form $(Fe^{+++}$. Theorell (1947) has shown that over the range pH 3.5 to 8, the difference between ferro- and ferri-cytochrome c remains constant at one equivalent per mole. Hence the oxidized cytochrome can be pictured as carrying one

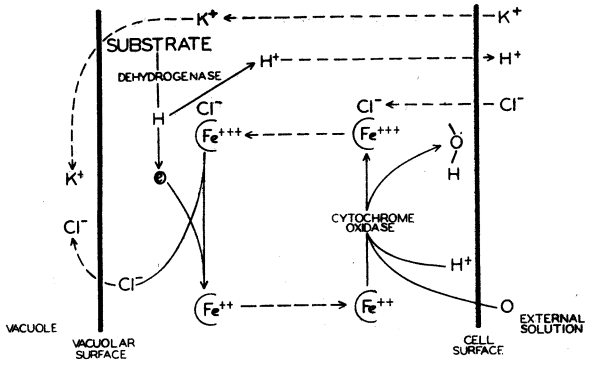

Fig. 5.—Schematic representation of the electron and anion transport system.

Solid lines represent chemical reactions; broken lines represent movements of substances; $(Fe^{+++}$, oxidized cytochrome; $(Fe^{++}$, reduced cytochrome.

positive charge more than the reduced form. The work of Theorell further indicates that this charge is associated through resonance with the imidazole nitrogen in the histidine of the cytochrome c protein. The oxidized cytochrome with excess positive charge will be capable of transporting an anion into the cell when it moves back to the reduction centre. This concept of movement between the oxidation and the reduction centres is introduced as an aid to the understanding of the hypothesis; the actual path may be very short.* When the cytochrome is reduced, the anion carried to the inside of the cell is left unpaired and will attract a cation. This cation could be a hydrogen ion, but since hydrogen ions readily exchange with cations from the external solution, the chances of it being a cation are high. The metallic cations can be pictured as entering by a series of exchanges along the hydrogen ion gradient; the net result would be the accumulation of both cation and anion towards the centre of the cell. This system can operate to accumulate ions only if it is assumed that the hydrogen ion path and the reduced cytochrome path are separated in the cell.

*Lundegardh (1945) has suggested that there may be an "electron wave;" this is alternative to the hypothetical movement of the redox substance, and could occur if the structural components of the cell contained electron conducting systems as suggested by Szent-Gyorgyi (1941).

Lundegardh (1940) has presented evidence for positive and negative areas in the cell surface, separated by regions of neutral molecules. It seems possible that the electron transport and the hydrogen ion paths may be associated with the extension into the cell of these different regions.

Provided that the electrons are fed to the cyclic system, anions will be conveyed through a spherical shell near the cell surface and accumulation will result. The measured rate of accumulation of anions will be the number conveyed by this system minus the number leaking from the cell by diffusion (Krogh 1946). If the leakage rate is low, at maximum accumulation the number of anions entering should approximately equal the number of electrons carried in the opposite direction. As pointed out in the introduction, the number of electrons passing through such a system will be four for each molecule of oxygen absorbed; the maximum rate of accumulation should be one molecule of monovalent salt accumulated for each electron eliminated in respiration, i.e. a ratio $\frac{\text{g. mol. salt accumulated}}{\text{g. mol. oxygen absorbed}}$ of 4.

(*b*) *Quantitative Relation between Salt Respiration and Salt Accumulation*

(i) *The Significance of the Ratio* $\frac{Accumulation}{Respiration}$.—The data from carrot tissue show that the salt accumulation (g. mol.) and the salt respiration (g. mol. oxygen absorbed) are of the same order of magnitude and that the ratio $\frac{\text{salt accumulation}}{\text{salt respiration}}$ ranges from low values for low concentrations to nearly four for high concentrations. Similar ratios are obtained in experiments with all the salts used; in the data available from other materials, ratios of the same order of magnitude are to be seen. The ratios of salt accumulated to oxygen utilized in salt respiration are close to the hypothetical at higher concentrations. Hence the results are not inconsistent with the Lundegardh hypothesis.

(ii) *Lower Ratios at Lower Salt Concentrations.*—Since it is only at the higher concentrations of salt that the ratios approach the theoretical, the cause of lower ratios at lower salt concentrations requires examination. The lower ratios are consequences of the relations of accumulation rate and salt respiration rate to salt concentration, as illustrated in Figure 2 which is based on the data in Table 2. The curve for salt respiration approximates to the asymptote at a much lower centration than does the curve for salt accumulation. Thus, in these experiments, salt respiration appeared to be almost at its maximum rate in a concentration of 0.02M while salt accumulation rate was still increasing with concentration. This means that the ratio for accumulation/respiration is less at lower concentrations. This observation is consistent in material from all sources and may be the explanation of Lundegardh's failure to obtain a stoichiometrical relationship between accumulation and respiration, since all his work appears

to have been done with comparatively dilute solutions. Lundegardh himself observed this effect of concentration (cf. Lundegardh 1940, p. 349) but placed on it a different intepretation from that given here.

(iii) *Variability in the Ratio.*—The high standard deviations (see Table 1) show the considerable variability which has been observed in ratios in different material at any one concentration of salt. The ratios will be affected by any variation or error in the estimate of either salt respiration or salt accumulation.

Estimates of salt respiration represent the difference between the total respiration in salt solution and the total respiration in water, which are both subject to errors of determination. Further, these estimates have been made on well-washed carrot tissue, in which the salt respiration is usually equal to the cyanide-sensitive respiration. In tissue which has been washed for shorter periods (up to 100 hours), a cyanide sensitive respiration, which appears soon after cutting, is still present. More investigation is required to determine how this cyanide-sensitive respiration is related to salt respiration; when this cyanide-sensitive respiration is at its maximum soon after cutting, addition of salt certainly gives no further increase. The significance of this, for present purposes, is that some persistence of this cyanide-sensitive respiration due to cutting would influence the estimate of salt respiration obtained experimentally, and Hanly and Turner (personal communication) have shown recently that the time for which this cyanide-sensitive respiration persists varies with the time of year at which the carrots are dug.

Variation in salt accumulation rate, which will also affect the ratio, may be due to the largely unknown factor of "leakage" or back diffusion from cells to solution. How far this is important, and how far it varies in different batches of carrot and in different salt concentrations, has yet to be investigated. Whatever the cause, it is certain that different sets of tissue can vary considerably in their accumulation rate at a particular concentration of salt; this is illustrated clearly in Figures 2 and 3. There is evidence that the rate of salt accumulation may be affected by time from cutting, because in Experiment C (Table 2) both salt respiration and salt accumulation increase with time from cutting over the range 144 to 312 hours. In Table 3 and in Figure 3, an increase in accumulation rate is shown between 120 and 168 hours from cutting; salt respiration did not change. Season of digging, age when dug, and time of storage of the carrots, may also have an influence. An important difference between material from different sources is illustrated in Figure 4. The Curve A, based on Table 1, shows that the ratio was still increasing at the highest concentration used, but Curves B and C, based on Table 3, show no increase in ratio at higher concentrations.

(iv) *Attainment of Hypothetical Value.*—Some factors may prevent the theoretical values for the ratio being observed. (*a*) ***Exchange effects.*** Increasing concentration externally would bring about increased ionic exchange (or re-

adjustment to new Donnan equilibria) with the cell contents; this would tend to allow entry of ions without the intervention of the accumulatory mechanism, and might therefore give false values for amount accumulated. Actually this may not be very important, particularly where the method of measuring the amount accumulated is by conductivity, since the ions entering by exchange are not recorded. If they were recorded, they would tend to make the $\frac{\text{accumulation}}{\text{respiration}}$ ratio high. (*b*) *Osmotic effects.* High concentrations of salt may cause the movement of water from the cells by osmosis. The amount of water withdrawn from the cell immediately after the salt is applied, and the effect of this withdrawal of water on the salt respiration and accumulation, has not yet been investigated. (*c*) *Permeability effects.* The nature and concentration of the salt applied externally are likely to have important effects on the permeability of the cell membrane to ions. They may effect both the entry of ions by the accumulatory mechanism (particularly as the cation must enter by a series of exchanges) and also the leakage or back diffusion from the cell. Leakage may be increased by increasing concentrations of monovalent ions externally because of the well-known increase in permeability resulting from monovalent cations replacing divalent cations, especially calcium, in the membrane. This leakage may therefore be enhanced and operate against the hypothetical ratio being obtained at higher concentrations (see later section).

(v) *Effects of Different Salts.*—The effect of divalent cations may be summarized by saying that they stimulate respiration and chloride accumulation but, some hours after their application, the rate of accumulation falls more than can be explained by increasing internal concentration (Robertson 1941). Comparison of dilute solutions of calcium chloride with potassium chloride is given in Tables 4 and 5. Here the ratios are similar for both salts but the ratio in more concentrated solutions is less in calcium chloride than in potassium chloride (see Table 6); the effect of the longer exposure may be seen in earlier work (Robertson 1941). Lundegardh's results for experiments extending over some hours show the ratios $\frac{\text{accumulation}}{\text{respiration}}$ in the following order Na > K > Mg > Ca > Sr > Ba, i.e. with the monovalents greater than the divalents. It seems possible that the lower ratios with higher concentrations or with prolonged exposure in the divalent salts are due to the divalent cations acting on the membrane, decreasing permeability, preventing the entry of further cations, and so slowing the rate of accumulation. Unequal absorption of anions and cations, with excess absorption of anions from divalent salts, has been known for some time. Ulrich (1942) has shown that excess anions are absorbed from solutions such as calcium bromide, and the cations which remain in excess in the external solution are balanced by an increase in bicarbonate ions. The failure of calcium ions to enter the cell could be due to the decreased permeability preventing exchange with hydrogen ions while the bromide is still

being accumulated. Accumulation of anions alone will not continue indefinitely, however; as Ulrich (1942) and Burstrom (1945) have shown, changes in the organic acid content occur within the cell, the acid content falling relative to the number of cations. This change in organic acid content may be related to the fall in respiration which is brought back to ground level and may even fall below it after the tissue has been in contact with the divalent cations for some hours (Robertson 1941).

Experiments on lithium chloride and sodium chloride in concentrations of 0.01M illustrate how closely salt accumulation rate is related to salt respiration rate. Though there is considerable variability between experiments, in any one experiment the respiration and accumulation by tissue in lithium chloride was always less than that in sodium chloride, averaging about two-thirds. However, the ratio of accumulation to salt respiration was the same in each. This indicates that at least in the range where salt respiration is limited by salt concentration, the accumulation rate is controlled by the salt respiration rate.

This discussion has been confined to halides because other ions are more likely to present complicated pictures. From Lundegardh's results, nitrate seems to be comparable to chloride, though there may be secondary effects with nitrate due to its participation in the cell metabolism. The differences in ratios obtained with nitrate, chloride, and sulphate may possibly be explained by differences in combining power of these ions with the cation (ferri-cytochrome) in the transport system. The observation that divalent anions such as sulphate are absorbed very slowly would be consistent with the difficulty of accumulating a divalent anion by a carrier geared to carry only one negative ion. Further, bicarbonate ions applied to the external solution would not be accumulated because it is unlikely that bicarbonate ions would exist in the acid environment of the cell in which the transport system is operating, unless there is a region of high alkalinity, which seems improbable.

(*c*) *The Respiratory Carrier: the Nature of the Salt Respiration*

While cytochrome and cytochrome oxidase are suggested as the substances responsible for the ion transport system, another electron carrier with similar characteristics would be just as suitable. The cytochrome system is suggested because (i) the accumulatory mechanism is stopped so readily by inhibitors of cytochrome oxidase, viz. cyanide, carbon monoxide, and azide (Lundegardh 1940; Machlis 1944; Robertson and Turner 1945); (ii) cytochrome represents the stage in the hydrogen transport system where the hydrogen ion and the electron become separated; and (iii) no other electron carrier has been shown to occur in carrot tissue.

The presence of a cytochrome oxidase in carrot tissue has been confirmed by isolation (Goddard, personal communication; Robertson and Bitmead, unpublished data). Attempts to isolate cytochrome c have been unsuccessful (Hanly and Turner, unpublished data). This does not necessarily imply absence

of cytochrome c, since its concentration may be quite low but still sufficient to keep pace with the comparatively low oxygen uptakes observed in plant tissue. This aspect of the work requires much more investigation. The general biological interest of the system in which a cyanide-sensitive respiration can be initiated by the addition of ions to the cell surface, has been the subject of previous comment; attention has been drawn to the similarity of this stimulated respiration to other types of stimulated respiration (Robertson and Turner 1945). It has been shown that the salt respiration is stimulated by the presence of salt in the external solution, is little affected by the salt content of the cell after accumulation, and takes a considerable time to disappear after removal of salt from the external solution. Further investigation of this stimulated respiration is suggested by the work of Goldinger and Barron (1946) in their investigation of the respiration of the fertilized sea-urchin egg. The stimulated respiration which occurs after fertilization is mediated by a cytochrome system. Goldinger and Barron suggested that the iron porphyrins are attached to structures containing highly polymerized nucleo-proteins and that the process of membrane formation is accompanied by an increase in permeability which will bring about a change in the electrolyte concentration, with subsequent depolymerization of the nucleoproteins and liberation of cytochromes. The possibility of a liberation of cytochromes after addition of salt to carrot tissue should be investigated.

The stimulation of salt respiration to a maximum occurs at concentrations of salt which are limiting to accumulation. This suggests that the stimulated respiration is due, not to the amount of salt being transported, but to some effect of salt on the respiratory system. This is borne out by the observation (Robertson and Thorn 1945) that the salt respiration persists for some time after accumulation has ceased due to removal of salt from the external solution. Further, it was shown (Robertson 1944) that a low concentration of salt (0.01M) would give a stimulated respiration with a high Q_{10} value, suggesting that the stimulated respiration was not limited by the entry of salt.

This observation introduces one difficulty in the Lundegardh hypothesis. If the hypothesis is correct, the fact that maximum salt respiration can be reached at concentrations below those necessary for maximum accumulation, would imply that some ion, other than one from the external solution, is available for the movement of the electron carrier in the oxidized condition (Fe^{+++}). This is not an insuperable difficulty since the production of some hydroxyl or other negative ion probably occurs at the oxidase, and negative ions may be transferred towards the centre of the cell with the ferri-cytochrome, finally combining with hydrogen ions. Production of such an ion would be analogous to the production of hydroxyl ions occurs in the "alkaline wave" of the gastric mucosa (see Davies 1946). The high concentration of an ion in the external solution necessary to bring the accumulatory mechanism to full rate could be pictured as being necessitated by the competition, for the positive centres on the carrier, between naturally produced negative ions and external anions.

(*d*) *The Steady State Condition of Accumulation*

The mechanism which has been discussed so far is that which transports the ions from the cell surface to the interior of the cell. The net accumulation rate which is measured will be the difference between the inward rate of ion transport by such a mechanism and the rate of loss or leakage of ions in the back diffusion from the interior of the cell towards the external solution. It seems probable that the rate of leakage will vary in different cells and in the same cell at different times. In any experimental work, the results represent the average behaviour of a large number of cells, and it is possible that some cells in the material, while operating to transport ions inwards may leak more rapidly than others. Further, since increase in permeability is a common accompaniment of death, it seems probable that ageing of tissue may be accompanied by an increase in the number of "leaky" cells.

Considerable evidence suggests that the permeability to ions of plant cell membranes is very low in healthy cells. This is shown particularly in the work on the electrical resistance of the surface of *Nitella* cells (Cole and Curtis 1938). Further, as Krogh has shown with radioactive ions, the resistance of the membrane to ionic movement in *Nitella* is very high. Indirect evidence of the magnitude of the leakage from cells of carrot tissue comes from the following observation: after accumulation of salt ceases, i.e. when the external solution is replaced with water, little solute escapes to the external solution although after about 70 hours the salt respiration drops to a low value, between 25 and 30 per cent. of the maximum value in 0.01M potassium chloride (Robertson and Thorn 1945). If it is assumed that the residual salt respiration just balances the leakage of ions, i.e. if it is assumed that each g. mol. of oxygen taken up is equivalent to about four anions which would have leaked and are being replaced, then the leakage would be of the right order of magnitude to account for discrepancies between the hypothetical and observed ratios. Lundegardh has suggested that leakage from wheat roots might account for the salt respiration "idling" in water. This is based on the observation that the respiration obtained by extrapolation to zero salt concentration is often lower than the actual respiration observed in water. On this picture the salt respiration "idling" is brought about by the leakage of ions from the cell and these ions are re-accumulated by this low salt respiration. Evidence for a possible similar effect is given by beet tissue (Robertson, Turner, and Wilkins 1947) in which the respiration in water was rarely fully cyanide insensitive, and by barley roots in which the respiration in water exceeds the cyanide stable respiration by about 60 per cent. of the latter. The possibility that leakage is greater in these tissues than in carrot and is associated with a residual or "idling" salt respiration could be investigated by prolonging the cyanide inhibition of accumulation to measure the leakage. Some leakage in cyanide has been observed in barley. Loss by back diffusion from the cells will contribute to departures from the hypothetical relationship and should be con-

sidered in any attempt to explain the form of the accumulation/time curves, particularly as the rate of leakage will increase as the internal concentration increases and the external concentration decreases.

(e) *The Lundegardh Hypothesis and Alternative Hypotheses*

The Lundegardh hypothesis has features in common with several earlier hypotheses. One suggestion (Brooks 1929, 1937) is that of an exchange of cations for hydrogen ions and of anions for bicarbonate ions. There are, however, a number of objections to this theory. Briggs (1930) pointed out that the suggested mechanism would not operate in a membrane with a mosaic of positive and negative areas but only in a membrane alternatively positive and negative in time. Even if this were assumed, there are other objections; because of the hydrogen ion characteristics of the cell surface, it is doubtful if there is much bicarbonate ion leaving the cell and it is likely that most of the carbon dioxide leaves as the molecule in solution. Even if exchange with the bicarbonate ion were possible, increased production of carbon dioxide alone is not capable of increasing accumulation. This is shown by experiments with methylene blue where carbon dioxide output is increased without any accompanying increase in accumulation rate (Hoagland and Broyer 1942; Briggs and Robertson, unpublished data). This may be very significant since the methylene blue may replace or by-pass the cytochrome system.

The total energy necessary to bring about accumulation at the observed rates is a very small fraction of that liberated in the salt respiration. Robertson (1941) has shown that the calculated energy required to effect accumulation from 0.01M potassium chloride was only about one per cent. of that freed. This low energy utilization is consistent with the view that the accumulatory mechanism is an incidental consequence of a particular respiratory mechanism. The system of a carrier as suggested by Lundegardh seems to be satisfactory to apply the energy to accumulation. It is similar in principle, though not in detail, to the one suggested by Wohl and James (1942). Lundegardh's hypothesis meets a number of the requirements imposed by other observations. For instance, it has been postulated that both the relation of accumulation rate to concentration and the changing rate of accumulation with internal concentration in time can be explained on the basis of a constant concentration layer at or near the surface of the cell (Robertson 1941). This layer would correspond to the saturated anion pick-up centres. The hypothesis also explains the observation by Lundegardh (1940) that the accumulatory mechanism can be inhibited by the application of electric current; electric current in the appropriate direction would prevent electrons from being moved to the surface and, therefore, as observed, would depress the salt respiration rate.

The hypothesis can also be applied to certain analagous processes in animal tissues. The possible applications were reviewed by Krogh (1946).

V. Conclusion

This paper presents evidence of the quantitative relation between salt accumulation and salt respiration; it is shown that the number of molecules of salt accumulated is of the same order of magnitude as the number of electrons eliminated in salt respiration; this in itself does not constitute proof of Lundegardh's hypothesis that the electron carrier of respiration behaves as an anion carrier, but is consistent with it. Since the hypothesis is in accord with other data it must be concluded that it is the most promising yet suggested. More work is necessary to show conclusively (a) that an electron carrier operates as the anion carrier and (b) that, if there is such a carrier, it is part of the cytochrome system.

VI. Acknowledgments

The work described in this paper was initiated in the Botany School, University of Sydney, and continued as part of the research programme of the Division of Food Preservation and Transport, C.S.I.R.

The writers wish to express their gratitude to Mr. R. C. Bitmead, Division of Food Preservation and Transport, C.S.I.R., who carried out the chloride determinations mentioned in Table 1; to the Professor of Botany, University of Sydney, and to Dr. Vickery, Chief of the Division of Food Preservation and Transport, C.S.I.R., in whose departments the experiments were carried out; and to Professor J. S. Turner, University of Melbourne, Professor J. G. Wood, University of Adelaide, and Dr. R. Lemburg, Institute of Medical Research, Royal North Shore Hospital, Sydney, for their helpful criticism of the manuscript.

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